Foetal deformities in a smooth-hound shark, *Mustelus mustelus*, from an oil exploited area in Angola

by

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ABSTRACT. - A smooth-hound shark, *Mustelus mustelus* (Linnaeus, 1758) caught off northern Angola had eight normally developed foetuses and one specimen with morphological abnormalities. This is the first teratologic embryonic deformation recorded for the species, presenting large scoliotic, lordotic and kyphotic bends in the vertebral column. All fins were present but malformed, and the eyes were well developed. The specimen had five pairs of gill slits and embryonic teeth on the mandible. Records of such abnormalities are rare, and the deformation ratio increases with environmental contamination. As the specimen was found in an extensive oil exploitation area the causality of the defects is discussed. Analyses of the toxic compounds in the sediments did not indicate pollution in the seabed, and selenium was the only compound found in relatively high levels (0.95 μg/g) in the muscle tissue of the embryo. Selenium is a naturally occurring trace element, which may cause teratogenic deformities if taken in excess; however, there is no statistical evidence to link the deformity to oil exploitation. Still, the record is important as it contributes to assess the frequency of teratogenic deformities in sharks, and in the long run, the effects of toxic components on embryo development.

RÉSUMÉ. - Malformations chez un fœtus de requin émissole, *Mustelus mustelus*, capturé dans une zone d'exploitation de pétrole en Angola.

Un requin émissole, *Mustelus mustelus* (Linnaeus, 1758), capturé au nord de l'Angola, avait huit fœtus normalement développés et un spécimen avec des anomalies morphologiques. C'est la première fois qu'une déformation tératologique embryonnaire est enregistrée chez cette espèce. La colonne vertébrale avait d'importantes courbures scoliotiques, lordotiques et cyphotiques. Toutes les nageoires étaient présentes, mais malformées, et les yeux étaient bien développés. Le spécimen avait cinq paires de fentes branchiales et des dents embryonnaires sur la mandibule. Les signalements de telles anomalies sont rares et le taux de déformation augmente avec la contamination de l'environnement. Comme le spécimen a été trouvé dans une zone d'exploitation pétrolière étendue, la causalité de ses anomalies embryonnaires est discutée. Les analyses des composés toxiques dans les sédiments n'ont pas indiqué de pollution des fonds marins, et seul le sélénium a été trouvé à des niveaux relativement élevés (0,95 μg/g) dans les tissus musculaires de l'embryon. Le sélénium est un oligo-élément naturel qui peut causer des effets tératogènes, lorsqu'il est en excès, mais il n'y a aucune preuve statistique pour lier la déformation observée à l'exploitation pétrolière. Néanmoins, ce type de signalement est important car il contribue à évaluer la fréquence des effets tératogènes chez les requins et, à long terme, les effets des composants toxiques sur le développement embryonnaire.

Key words. - Triakidae - *Mustelus mustelus* - Angola - Deformity - Heavy metals - Selenium - Teratology.

Deformity is rarely reported in elasmobranches (Heupel *et al*., 1999), but it is known that different kind of deformities occur in adults (Bensam, 1965; Hoenig and Walsh, 1983; Pastore and Prato, 1989; Officer *et al.,* 1995; Brahim and Capapé, 1997; Heupel *et al*., 1999; Teshima *et al.,* 1999; Coelho and Erzini, 2006; Mancini *et al.,* 2006; Preziosi *et al*., 2006), and is occasionally found in embryos (Bensam, 1965; Goto *et al*., 1981; Bonfil, 1989; Pastore and Prato, 1989; Ferreira *et al.,* 2000, 2002; Mancini *et al.,* 2006; Galván-Magaña *et al*., 2011). Generally, the direct cause of abnormalities in elasmobranches is difficult to identify, but possible causes of skeletal deformities in this group may include ¨asymmetrical stresses to the vertebral column, parasites, arthritis, injury, disease, localized tumours or malnutrition" (Heupel *et al*., 1999). Spinal deformity in fish may be related to contamination of heavy metals (Bengtsson *et al*., 1988; Messaoudi *et al*., 2009) and the rate of deformed embryos and larvae increases with the level of contamination (Lemly, 1998, 2002a, 2002b). Despite the increased mortality risk for deformed fish, observations of adult wild fish with such deformities are not uncommon in polluted areas (Messaoudi *et al*., 2009). Sharks are typically top predators and can accumulate high concentrations of toxic compounds (Storelli *et al.,* 2003), but the low number of offspring for viviparous sharks in combination with the ethical and the practical difficulties associated with catching a high number of pregnant sharks make experimental designs used for teleosts not suitable. Instead, qualitative methods may in the long run provide an aggregation of observations which may indicate the causality to examining the effect of toxic components on embryo development in sharks.

The smooth-hound, *Mustelus mustelus* (Linnaeus, 1758),

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is a common demersal shark, distributed in the continental shelves and upper slopes throughout the eastern Atlantic: from the United Kingdom to the Mediterranean, down to Morocco, Canary Islands, and further south to South Africa (Compagno *et al*., 2005). Despite a large distribution area, large migrations are not reported. It typically feeds on benthic crustaceans, but also on cephalopods and bony fishes. It is a viviparous shark with yolk-sac placenta, producing 4 to 15 litters (Compagno, 1984; Compagno *et al*., 2005). Its feeding behaviour exposes individuals of *M. mustelus,* inhabiting environmental contaminated areas, to bioaccumulation.

This paper reports for the first time a teratologic embryonic deformation in *M. mustelus*, and the availability of samples of sediments and muscle tissue of the embryo made it possible to perform toxic components analyses and to discuss possible causes for the deformation.

MATERIALS AND METHODS

In Angola, bottom trawl surveys have been conducted each year since the beginning of the 1990's with the R/V "Dr. Fridtjof Nansen" (Sætersdal *et al*., 1999). Sharks received little attention in earlier surveys, but in the period 2002-2010 over 5000 specimens of sharks have been identified to species and undergone biological and morphometric measurements (Zaera, unpubl. data). In the morning 28 March 2006, one *M. mustelus* was caught in a bottom trawl (7°10'S; 12°16'E, 118 m depth) in the vicinity of the rich oil exploitation block 4 off N'zeto (Fig. 1). Upon dissection the specimen was found to contain nine foetuses of which eight showed no sign of abnormality, whereas one was markedly deformed (Fig. 2). The malformed embryo was measured and digital pictures were taken. At the University of Bergen, the specimen was X-rayed with an intensity of 10 mA, using different exposure times (0.10, 0.12, 0.16, 0.18, 0.22, 0.6 and 0.8 s).

Two weeks later, and by coincidence, (14-17 April 2006) an environmental survey with R/V "Dr Fridtjof Nansen" was carried out in the oil exploited area off Northern Angola. Based on a request from the authors, four sediments samples were taken with a Van Veen grab in the vicinity of the position of the catch of the *M. mustelus* specimen (see Fig. 1 for station positions). A standard inductively coupled plasma – atomic emission spectrometry was used to analyse barium (Ba), copper (Cu), chromium (Cr), zinc (Zn), lead (Pb) and cadmium (Cd). Later, a 197 multi-element inductively

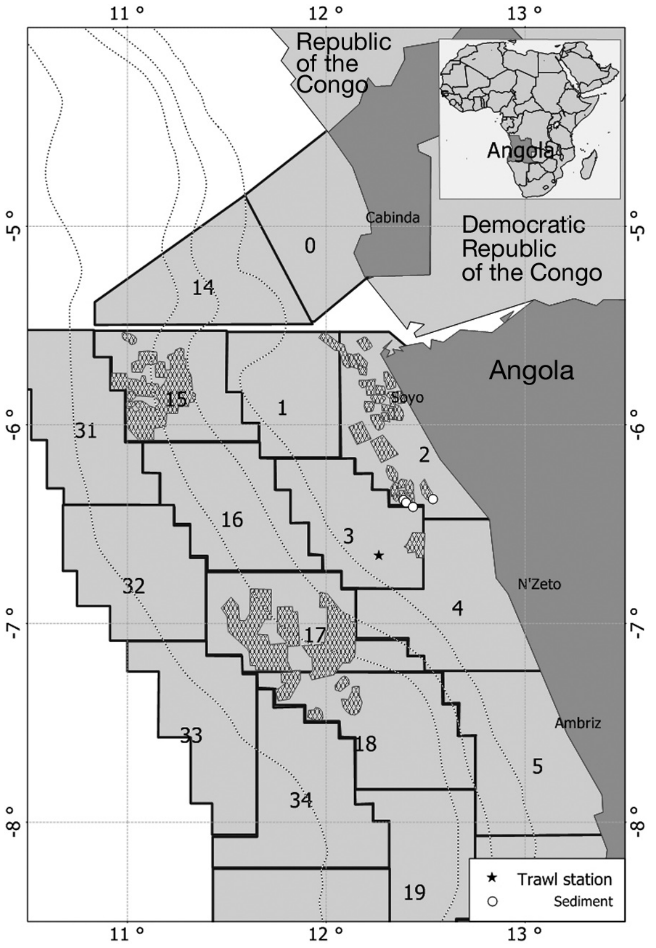

Figure 1. - Map showing position of captured shark, *Mustelus mustelus*, location of sediment samples, environmental stations and oil exploitation areas (hatched), off northern Angola.

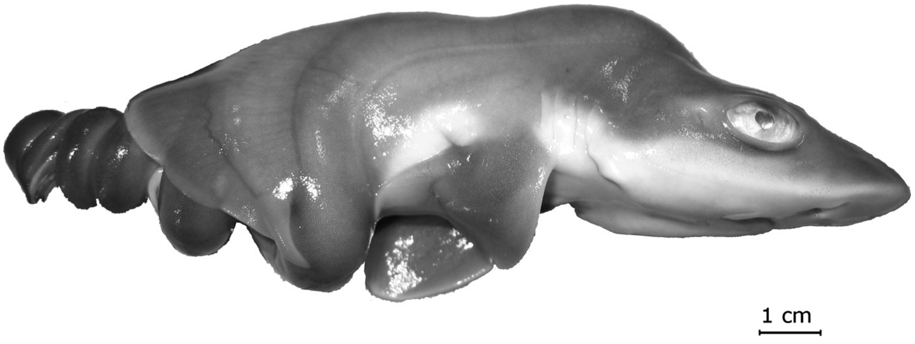

Figure 2. - Deformed *Mustelus mustelus* foetus in lateral view showing a rolled clockwise body. Scale bar = 1 cm.

coupled plasma mass spectroscopy was used to analyse trace element concentrations: arsenium (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) in the muscle tissue of the shark at the Norwegian National Institute of Nutrition and Seafood Research (NIFES).

RESULTS

The abnormal embryo had large scoliotic, lordotic and kyphotic bends in the vertebral column. The trunk of the malformed foetus appears to have suffered a linear shortening "creating" a hump-like structure right behind the head (Figs 2, 3). The rest of the trunk was rolled clockwise just behind the pelvic fins and until the caudal fin. There were hardly any straight segments (Fig. 3). The fins were all present but malformed (Fig. 2). The most relevant head's external features were normal: eyes were well developed, five pairs of gill slits were present on each side of the head and the mandible had embryonic teeth. Claspers were visible. Based on the embryos' size (born at around 39 cm total length, *TL*), pigmentation and the presence of an umbilical peduncle they were mid-term foetuses. The morphometric measurements of the malformed embryo are presented in table I.

Mean values for the heavy metals measured in both the sediments and the muscle tissue of the abnormal embryo are presented in table II. In the sediments, Zn had the highest value followed by Cr, Ba, Pb, Cu and Cd. In the muscle tissue, As was found in highest concentration followed by Se, Pb, Hg and Cd. As trace metal measurements in the sediments and the muscle tissue followed different standard procedures, only Cd and Pb were measured in both, and their concentrations were 41 and 74 times higher in the sediments than in the tissue respectively (Tab. II). The concentrations measured in the sediments (Tab. II) are moderate and in most cases under the level of significant contamination (Anonymous, 2006, 2008). Also the tissue concentrations of As, Cd, Hg and Pb seem moderate, while the value of selenium (0.95 μg/g wet weight) may be classified as high based on literature values (Lemly, 2002a; Paul *et al.*, 2003; Branco *et al.*, 2007; Jasonsmith *et al.*, 2008).

The temperature, salinity and oxygen content were 16.35°C, 35.68 PSU (Practical Salinity Units) and 1.45 ml/l (26% of saturation), respectively, 10 m above the seabed at the station where the smooth-hound was caught.

Table I. - Morphometric measurements (in mm) of an abnormal embryo of *Mustelus mustelus* as absolute measurements (AV) and as percentage of total length (RE), for both sides. All values are in mm except total weight (TW) in g.

	AV		RE	
	Right	Left	Right	Left
TL (total length)	120			
POB (preorbital length)	20	19	16.67	15.83
PSP (prespiracular length)	31	34	25.83	28.33
PG1 (prebranchial length)	38	43	31.67	35.83
HDL (head length)	47	48	39.17	40.00
PD1 (pre-first dorsal-fin length)	63	68	52.50	56.67
PP1 (prepectoral-fin length)	41	44	34.17	36.67
PRN (prenarial length)	9	17	7.50	14.17
POR (preoral length)		17		14.17
EYL (eye length)	10	12	8.33	10.00
EYH (eye height)		5		4.17
GS1 (first gill slit height)	8	6	6.67	5.00
GS5 (fifth gill slit height)	5	5	4.17	4.17
ING (intergill length)	10	10	8.33	8.33
P1B (pectoral-fin base)	10	8	8.33	6.67
P1A (pectoral-fin anterior margin)	30	29	25.00	24.17
P1P (pectoral-fin posterior margin)	20	21	16.67	17.50
P1I (pectoral-fin inner margin)	13	15	10.83	12.50
D1B (first dorsal-fin base)		26		21.67
D1A (first dorsal-fin anterior margin)		33		27.50
D2B (second dorsal-fin base)		23	19.17	19.17
D2A (second dorsal-fin anterior margin)		20	16.67	16.67
D1P (first dorsal-fin posterior margin)		32		26.67
D1I (first dorsal-fin inner margin)		8		6.67
HDH (head height)		24		20.00
TRH (trunk height)		23		19.17
INW (internarial space)		9		7.50
MOL (mouth length)		8		6.67
MOW (mouth width)		14		11.67
LLA (lower labial furrow length)		4		3.33
ULA (upper labial furrow length)		5		4.17
INO (interorbital space)		20		16.67
SPL (spiracle length)	1	1	0.83	0.83
ESL (eye spiracle space)	2	3	1.67	2.50
HDW (head width)		24		20.00
TRW (trunk width)		17		14.17
ABW (abdomen width)		10		8.33
CPW (caudal-fin peduncle width)		3		2.50
TW (total weight)	49.5			

DISCUSSION

For the first time, a teratologic embryonic deformation is reported in *M. mustelus*, and only a few records of similar malformations in other shark species are known (Pastore and

Table II. - Heavy metal levels in the abnormal *Mustelus mustelus* embryo body tissue and in the sediments sampled (STD values in brackets) off Angola (N = 4). Values are presented as μg/g of wet weight. * 22.9 % dryness.

	As	Cd	Hg	Pb	Se	Ba	Cu	Cr	Zn
* Tissue	2.9	0.01	0.03	0.06	0.95				
Sediments		0.41		4.43		22.58	1.25	25.45	30.55
		(0.10)		(2.12)		(30.53)	(1.08)	(8.75)	(13.72)

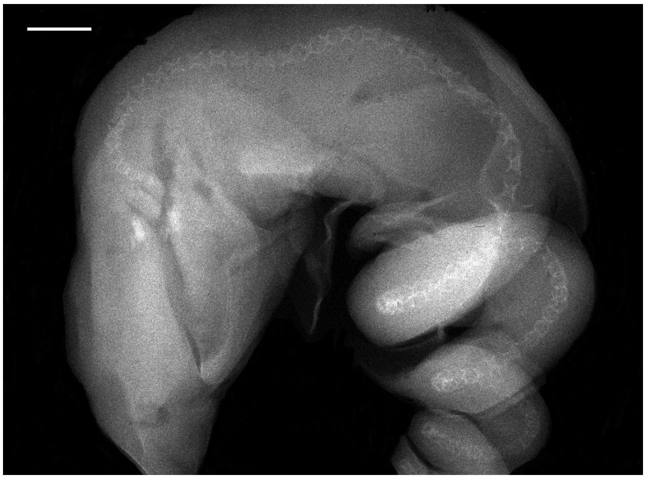

Figure 3. - X-ray of the *Mustelus mustelus* foetus in lateral view. Exposure is 12 s with 10 mA. Scale bar = 1 cm.

Prato, 1989; Mancini *et al*., 2006). The few published observations suggest a low occurrence of such deformations, but little is known about the reasons or frequency of occurrence. The pregnant mother was caught in an oil exploited area, and available data from an environmental study in the temporal and spatial vicinity of the trawl station provided a unique opportunity to examine potential reasons for the deformity. To further investigate the causality, the muscle tissue of the embryo was analysed for heavy metals. Sharks are not targeted during the demersal surveys off Angola, and are typically identified to species, counted and weighted. Unfortunately, by the time the importance of the finding was clear, the mother and the siblings had been discarded and only the deformed embryo was kept for further analyses. The fact that the demersal and the environmental surveys belonged to two different unrelated projects, generated differences in the components measured in the muscle tissue and the sediments, weakening the study.

Although in this paper the cause of the abnormality has been linked to pollution, the authors are aware that other factors as genetic, malnutrition, injury and parasites may cause deformation (Heupel *et al*., 1999). Although the concentrations of heavy metals measured in the sediments and in the tissue were moderate (Lemly, 1985; Storelli *et al*., 2003; Anonymous, 2006; Branco *et al*., 2007; Anonymous, 2008; Escobar-Sánchez *et al*., 2010), the value of selenium in the embryo's muscle tissue was moderate-high in comparison to published data (Storelli and Marcotrigiano, 2002; Escobar-Sánchez *et al*., 2010). At the present, the available data cannot provide clear evidences for the cause of the deformation, but in spite of the role of selenium as an essential micronutrient, it has become a primary element of concern in many environmental contamination situations due to its bioaccumulative nature causing, otherwise harmless concentrations of selenium, to reach toxic levels (Lemly, 2002b; Hamilton, 2004). Selenium is known to have a very narrow hormetic curve, being toxic above a certain amount causing neurologic, endocrine and dermatologic problems (Lemly, 1998; Gerstenberger *et al*., 2010). Furthermore, due to its mobility through the placenta, pregnant females and their foetuses can be considered as a risk group in the presence of high concentrations of selenium (Lemly 1998; Gerstenberger *et al*., 2010). For many teleosts the deformities during larval ontogeny are found to increase with high concentration of selenium (Lemly, 2004), and sharks as typical top predators can accumulate large concentrations of toxic compounds (Storelli *et al*., 2003). When feeding near or on the seabed, primarily on benthic crustaceans (Compagno, 1984; Compagno *et al*., 2005), the potential to accumulate heavy metals seems large for *M. mustelus*.

In the Arabian Gulf, an area with heavy oil exploitation activity and large spills of crude oil, several shark species show bioaccumulation of hydrocarbon pollution (Al-Hassan *et al.,* 2000). They found two fully developed foetuses of blacktip shark, *Carcharhinus limbatus*, with no apparent deformities and with the same level of Polycyclic Aromatic Hydrocarbons (PAHs) as the mother. Hopkins *et al*. (2006), working with toads, found out that mothers transfer significant amounts of selenium to their eggs with the consequent reduction of offspring viability, demonstrating that maternal transfer may be an important route of contaminant exposure in amphibians. It is possible that the same occurs in viviparous sharks and the selenium level in the smooth-hound foetus is about the same level as for the mother.

Although some authors have pointed out both genetic and environmental factors as possible causes for foetal deformities (e.g., Schultz, 1963; Bengtsson *et al.,* 1988) and heavy metals like cadmium, lead and selenium (e.g., Bengtsson, 1975; Newsome and Piron, 1982; Bengtsson *et al.,* 1985; Lemly, 2002a) as possible cause to skeletal abnormalities, only a very few studies have investigated the effects of pollutants on the wellbeing and health of sharks (McClusky, 2006) or link the observation of morphological deformities to the surrounding environment (Al Hassan *et al*., 2000; Storelli and Marcotrigiano, 2002; Storelli *et al.,* 2003). Although, it is clearly impossible to conclude on the reason for the morphological deformities in the presented embryo, environmental contamination cannot be discarded as a possible cause for the observed deformity.

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